DAIRY FOODS

Proteolysis and Rheology of Low Fat and Full Fat Mozzarella Cheeses Prepared from Homogenized Milk[1]

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ABSTRACT

The rheological and proteolytic characteristics of low fat and full fat Mozzarella cheeses made from milk homogenized at 10,300 and 17,200 kPa were compared with those of cheeses prepared from nonhomogenized milk. Half of the samples were cooked at 45.9°C and half at 32.4°C; the lower temperature resulted in higher moisture in nonfat substance. α_{s1}-Casein partially degraded to α_{s1}-I-casein in the cheeses cooked at the lower temperature during 6 wk of refrigerated storage. Except for the 17,200-kPa cheese, proteolysis was dependent on moisture in nonfat substance. Hardness increased with homogenization pressure and decreased with fat percentage and moisture in nonfat substance. Meltability was aided by storage and hindered by fat reduction, higher cooking temperature, and homogenization. Storage modulus decreased during storage and increased with pressure and cooking temperature. A low fat Mozzarella having textural and melting properties comparable with those of a normal high fat cheese can be prepared using homogenized milk, a lower preparation temperature, and refrigerated storage.
(**Key words**: homogenization, Mozzarella cheese, proteolysis, rheology)

Abbreviation key: **HF** = high fat, **HT** = high temperature, **LF** = low fat, **LT** = low temperature, **MNFS** = moisture in nonfat substance.

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[1]Mention of brand or firm names does not constitute an endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

INTRODUCTION

Recent research (20) has shown a significant correlation between dairy fat intake and mortality rates from coronary heart disease. Such studies have caused American consumers to become more concerned about their consumption of fat in cheese and other dairy products. Mozzarella cheese is currently being consumed at a per capita rate of 3.2 kg/yr in the US and is exceeded only by Cheddar cheese in popularity (25). Part-skim Mozzarella normally contains 16 to 17% fat (24); production of such cheese containing less fat is of interest. Previous reports from this laboratory (22, 23) described the improvement of textural and melting characteristics in Mozzarella containing 9 to 11% fat. Because homogenization of milk is desirable in some cheese-making applications, such as utilization of recombined milk, the use of homogenized milk in the preparation of low fat (LF) and full fat [high fat (HF)] Mozzarella was investigated.

Homogenization has been previously used to improve the yield of Mozzarella (2, 18). Lelievre et al. (15) found that low pressure homogenization decreased stretchability and meltability of Mozzarella; they attributed this effect to the presence of casein at the water-fat droplet interface, which causes the fat droplets to be crosslinked in the protein matrix. The effect of different homogenization pressures and temperatures on rheology, meltability, and other characteristics of buffalo milk Mozzarella has also been investigated (9). However, no such studies had been conducted on LF Mozzarella prepared from homogenized cow's milk from cows. Protein degradation in Mozzarella during refrigerated storage has been studied (3, 4, 5, 22), but proteolysis in Mozzarella made from homogenized milk has not been examined electrophoretically. In this paper, we compare rheology, meltability, and proteolysis of LF and HF Mozzarella cheeses prepared from homogenized and nonhomogenized milks.

MATERIALS AND METHODS

Cheese Preparation

The LF and HF Mozzarella cheeses were prepared as described by Tunick et al. (22). Each type of cheese was prepared from 22.7 kg of milk, and one batch was prepared on a given day. Each batch was standardized with cream or skim milk to the desired fat percentage prior to pasteurization at 63°C for 30 min. The LF cheese milk was standardized to 1.0% milk fat and the HF cheese milk to 3.5% milk fat. The milk used for homogenized cheeses then underwent two-stage homogenization at 63°C at pressures totaling 10,300 and 17,200 kPa. The designation of 0 kPa was used for nonhomogenized milk. Cheese milk at 32.4°C was inoculated with 125 ml of CR7 starter culture (50% *Streptococcus salivarius* ssp. *thermophilus* and 50% *Lactobacillus bulgaricus*; Marschall Products Rhône-Poulenc Inc., Madison, WI). After the pH decreased .1 unit, 4.4 g of single-strength calf rennet (Chr. Hansen's Lab., Inc., Milwaukee, WI) were added. The curd was held for 35 min, cut, and held for another 15 min. High temperature (HT) cheeses were heated over 45 min to 45.9°C and held for 50 min. Low temperature (LT) cheeses were stirred at 32.4°C for 10 min and held at that temperature for 90 min. The whey (pH 6.3 to 6.4) was then drained, and the curd was rinsed and cut into slabs. When the pH declined to 5.2 to 5.3, the slabs were covered and iced overnight. The next day, the curd was divided into eight parts and stretched and kneaded multidirectionally by hand for 7 min in water at 70 to 80°C. The samples were pressed into 224-ml polyethylene cups (approximately 80 mm in diameter and 55 mm high), cooled, removed from the cups, brined for 2 h in 23% salt solution, blotted dry with clean paper towels, and stored in vacuum sealed pouches at 4°C for up to 6 wk. Two cheese types were prepared each week over a period of several months according to a 3 × 2 × 2 factorial arrangement in a completely random design. The types consisted of LF, HT; LF, LT; HF, HT; and HF, LT at each of the three homogenization pressures; there were four replicates of each type at 0 kPa and two replicates of each type at 10,300 and 17,200 kPa. Each sample was analyzed after 1, 3, and 6 wk of storage.

Electrophoresis

Cheese samples were extracted for protein analysis at 1, 3, and 6 wk with a pH 8.0 solution of .166 *M* Tris (Sigma Chemical Co., St. Louis, MO), 1 m*M* EDTA (Fisher Scientific, Pittsburgh, PA), 2.9% SDS (Sigma Chemical Co.), and 1.7 m*M* dithiothreitol (Calbiochem, San Diego, CA), as described by Tunick et al. (22). Lyophilized extracts were stored at –20°C. The SDS-PAGE of extracts was performed with the PhastSystem® (Pharmacia, Piscataway, NJ) using 20% homogeneous gels. Gels were stained with a .1% solution of Coomassie blue R250, destained, and dried. A Bio-Rad model 620 Video Densitometer (Richmond, CA) interfaced with a computer and 1D Analyst II (Version 3.10) software (Bio-Rad), was used to scan the gels and to integrate peak areas. Characterization and quantitative analysis of peptides were not included in the calculations.

Rheological Analyses

Texture profile analysis was performed at 1 and 6 wk as previously described (22); hardness and springiness were determined at 23 to 26°C using an Instron Universal Testing Machine (model 4201; Instron, Inc., Canton, MA). After tempering at these temperatures for 1 h, a slab of cheese was removed from the interior of the sample by cutting with piano wire held in a frame. Four to six cylindrical specimens (approximately 14 mm in diameter and 14 mm high) were then removed from the slab using a cork borer. Slabs were removed at different angles relative to the axis of the cheese cylinder to minimize effects of curd orientation. The storage modulus and loss modulus were determined at 1 and 6 wk with a Rheometrics Dynamic Analyzer RDA-700 (Rheometrics, Inc., Piscataway, NJ) at 23 to 26°C at a frequency of 100 rad/s at .8% strain (22). Three disks (25.4 mm in diameter and 4 to 5 mm thick) were removed from the interior of the cheese by piano wire and cork borer and glued with cyanoacrylate bonding agent to pairs of parallel aluminum plates for the analyses.

Other Analyses

The moisture content of the samples was measured by the forced-draft oven method (1), and fat content was determined by the modified Babcock test (12). These analyses were performed after 1 wk. The percentage of NaCl was determined for some samples in triplicate by titration with .2*N* $AgNO_3$ using 2% K_2CrO_4 as the indicator. Meltability was determined at 1 and 6 wk by the Schreiber test, in which the expansion of a disk of cheese (18 mm in diameter, 5 mm thick) is measured on a target graph of concentric circles after 5 min in an oven at 232°C (12, 17); e.g., meltability of 1.0 indicates no expansion, 2.0 an expansion of 5 mm, and 3.0 an expansion of 10 mm. Three replicates of each sample were analyzed.

The data were analyzed by the general linear models procedure of SAS (21). The compositional data were analyzed by factorial analysis of variance to examine the effects and interactions of fat, temperature, and pressure. Rheological and electrophoretic responses were analyzed similarly, but time also was included. An interaction was described as significant only when $P < .05$. Linear regressions were performed on some of the hardness and meltability data.

RESULTS AND DISCUSSION

Cheese Making and Composition

Curd shattering during stirring was evident when cheeses were prepared from homogenized milk. Finely shattered curd particles gave the whey a cloudy appearance, although the yield of homogenized cheese was still greater than that of the nonhomogenized cheese. Most cheeses had a pH of 5.25 to 5.35 before stretching, although some had a pH as low as 5.10. During stretching, the 10,300- and 17,200-kPa cheeses exhibited much less oiling off and a shorter body than the 0-kPa cheeses. Homogenized cheeses were white in appearance, whereas the nonhomogenized cheeses were light yellow. The average percentage of NaCl in all types of cheese was 1.10 ± .13. Other compositional analyses, including moisture in nonfat substance (MNFS), are shown in Table 1. A statistical summary is given in Table 2.

Proteolysis

Significant amounts of proteolysis of α_{s1}-casein occurred during refrigerated storage of the LT cheeses. Rennet, which degrades α_{s1}-casein, retains some activity during Mozzarella preparation (4, 22). Initial cleavage of α_{s1}-casein by rennet results in the formation of α_{s1}-I-casein, the large peptide remaining after the loss of residues 1 to 23. Table 3 shows the percentages of α_{s1}- and α_{s1}-I-caseins present in the cheeses during storage. The percentages of α_{s1}-I-casein in the samples at 1 wk were negligible and are not shown.

Although the optimal temperature for rennet activity is about 45°C (6), cooking of cheese at this temperature resulted in insignificant α_{s1}-casein degradation during storage compared with that of cheeses prepared at 32.4°C. As a result, temperature × week interaction with α_{s1}-casein and α_{s1}-I-casein was significant (Table 2). The difference in proteolysis at the two temperatures was evidently due to the higher MNFS in the LT cheeses than in the HT cheeses. The calculation of MNFS, because it excludes fat, is essentially a ratio of water to protein. An increase in MNFS increases the rate of proteolysis in cheese (14). However, the lower cooking temperature did not lead to significant α_{s1}-casein proteolysis in the 17,200

TABLE 1. Compositional analyses of Mozzarella cheeses.

Type[1]	Moisture	Fat in DM	MNFS[2]
	(%)		
	0 kPa		
LF, HT	52.5	23.3	59.0
LF, LT	54.1	22.6	60.4
HF, HT	46.3	48.2	62.4
HF, LT	49.5	47.2	65.2
	10,300 kPa		
LF, HT	55.1	20.9	60.8
LF, LT	56.6	21.3	62.4
HF, HT	47.9	51.7	65.5
HF, LT	48.2	51.0	65.5
	17,200 kPa		
LF, HT	53.9	23.5	60.5
LF, LT	56.8	24.7	63.7
HF, HT	46.8	52.2	64.8
HF, LT	47.0	51.3	64.6

[1]HF = High fat, HT = high temperature, LF = low fat, LT = low temperature.

[2]MNFS = Moisture in nonfat substance.

kPa cheeses, possibly as a result of physical changes to the casein at the high homogenization pressure.

The average α_{s2}-casein content in all cheeses was 12.6 to 16.0% at 1 wk and 11.2 to 14.7% at 6 wk. The average β-casein content in all samples was 36.4 to 42.6% at 1 wk and 34.1 to 39.7% at 6 wk. No significant variations were attributable to fat content, cooking temperature, or homogenization pressure. Both types of casein are resistant to the action of rennet (6), and the small amount of degradation observed may have been due to plasmin, a heat-stable protease in milk (5, 6). This effect has been noted in Mozzarella in a previous study by this laboratory (22). More extensive proteolysis of α_{s2}- and β-caseins in Mozzarella has been observed by others (5), but different coagulants were used. The *L. bulgaricus* and *S. salivarius* ssp. *thermophilus* in various starter cultures exhibit proteolytic activity (19), and both cause casein to be hydrolyzed (10). These bacteria were used in the present study and in an earlier investigation (22) in which both types were present in Mozzarella after 6 wk of refrigerated storage. The starter bacteria may account for some of the proteolysis observed during the 6-wk period.

Hardness and Springiness

Hardness increased with cooking temperature (Table 4). The HT cheeses contained less MNFS than their LT counterparts, leading to

TABLE 2. Statistically significant factors and interactions in Mozzarella cheeses.

		MNFS[2]		Hardness		Springiness[3]		Meltability	
Source[1]	df	MS[4]	*P*	MS	*P*	MS	*P*	MS	*P*
Temperature (T)	1	21.62	<.0001	19,570	<.0001			.3906	.0092
Fat in DM (F)	1	107.7	<.0001	57,780	<.0001	10.33	<.0001	6.250	<.0001
T × F	1			5910	.0005				
Homogenization pressure (P)	2	11.36	.0037	5178	<.0001			7.137	<.0001
T × P	2			1369	.0473				
F × P	2					1.768	.0047	5.982	<.0001
Weeks of storage (W)	1					26.19	<.0001	19.13	<.0001
T × W	1					1.653	.0189		
F × W	1					2.413	.0056		
P × W	2							.2913	.0072
Residual	40	1.511		415.4		.2589		.0521	

		Storage modulus		α_{s1}-Casein[5]		α_{s1}-I-Casein[5]	
		MS	*P*	MS	*P*	MS	*P*
T	1	28.88	.0052	713.3	.0004	853	<.0001
F	1			403.0	.0064		
T × F	1						
P	2	27.03	.0011				
T × P	2			208.3	.0208		
F × P	2	18.73	.0068				
W	1	19.13	.0209	1062	<.0001	1270	<.0001
T × W	1			472.3	<.0003	128.4	.0336
F × W	1						
P × W	2						
Residual	40	3.305		49.76		35.32	

[1]Effects and interactions of cooking temperature, fat in DM, homogenization pressure, and weeks of storage tested against residual error.

[2]Moisture in nonfat substance. Residual df = 20.

[3]Residual df = 23.

[4]Type I mean squares.

[5]Residual df = 50.

TABLE 3. Effect of homogenization on percentage of α_{s1}-caseins present in Mozzarella cheese samples.[1]

Type[2]	α_{s1}-Casein[3] 1 wk	3 wk	6 wk	α_{s1}-I-Casein[3] 3 wk	6 wk
			0 kPa		
LF, HT	46.8	44.5	46.6	4.4	6.0
LF, LT	45.2	35.9	24.0	8.9	22.4
HF, HT	39.8	37.9	33.1	4.5	13.8
HF, LT	40.4	29.4	18.9	18.2	23.6
			10,300 kPa		
LF, HT	47.0	46.5	39.1	2.6	9.9
LF, LT	45.0	29.6	22.5	15.9	27.0
HF, HT	44.2		39.7		7.8
HF, LT	43.0	40.9	18.4	6.7	29.0
			17,200 kPa		
LF, HT	43.8	39.7	42.3	6.1	10.0
LF, LT	57.3	48.1	43.7	5.7	4.2
HF, HT	45.8	41.5	39.7	0	12.5
HF, LT	47.3	43.7	31.6	2.0	16.3

[1]Data obtained from densitometry measurements of SDS-PAGE on 20% homogeneous gels. Percentages based on total amount of α- and β-caseins.

[2]HF = High fat, LF = low fat, HT = high temperature, LT = low temperature.

[3]Average of 4 replicates in 0-kPa cheeses; average of 2 replicates in 10,300- and 17,200-kPa cheeses.

TABLE 4. Effect of homogenization on hardness, springiness, meltability, and storage modulus of Mozzarella cheeses.

Type[1]	MNFS[2]	Hardness[3] 1 wk	6 wk	Springiness[4] 1 wk	6 wk	Meltability[5] 1 wk	6 wk	Storage modulus[6] 1 wk	6 wk
	(%)	(N)		(mm)				(N/cm^2)	
					0 kPa				
LF, HT	59.0	127	119	9.38	8.33	.9	1.6	8.65	6.19
LF, LT	60.4	96	90	8.78	7.62	1.3	1.5	6.28	6.23
HF, HT	62.4	55	45	8.04	6.42	2.4	3.0	5.45	5.34
HF, LT	65.2	44	26	8.14	5.18	2.7	3.2	4.20	3.16
					10,300 kPa				
LF, HT	60.8	137	143	9.01	7.71	1.2	1.4	7.85	7.16
LF, LT	62.4	83	67	9.06	7.14	1.2	1.7	6.31	4.39
HF, HT	65.5	76	68	8.31	6.93	1.0	.9	8.14	6.98
HF, LT	65.5	60	51	8.19	6.15	1.1	1.2	8.03	6.54
					17,200 kPa				
LF, HT	60.5	193	194	7.62	7.57	.9	1.4	6.31	8.88
LF, LT	63.7	119	74	7.81	7.34	1.1	1.3	7.00	4.66
HF, HT	64.8	82	77	7.55	6.44	1.1	.9	10.9	5.70
HF, LT	64.6	56	68	8.86	6.55	1.0	.9	7.71	8.44

[1]HF = High fat, HT = high temperature, LF = low fat, LT = low temperature.

[2]Moisture in nonfat substance. Average of 12 replicates in 0-kPa cheeses; average of 6 replicates in 10,300- and 17,200-kPa cheeses. Root mean square for error (RMSE) = 1.23 with 20 df.

[3]Average of 16 replicates in 0-kPa cheeses; average of 8 replicates in 10,300- and 17,200-kPa cheeses. RMSE = 20.4 with 40 df.

[4]Average of 16 replicates in 0-kPa cheeses; average of 8 replicates in 10,300- and 17,200-kPa cheeses. RMSE = .51 with 23 df.

[5]Average of 12 replicates in 0-kPa cheeses; RMSE = .23 with 40 df.

[6]Average of 12 replicates in 0-kPa cheeses; average of 6 replicates in 10,300- and 17,200-kPa cheeses. RMSE = 1.82 with 40 df.

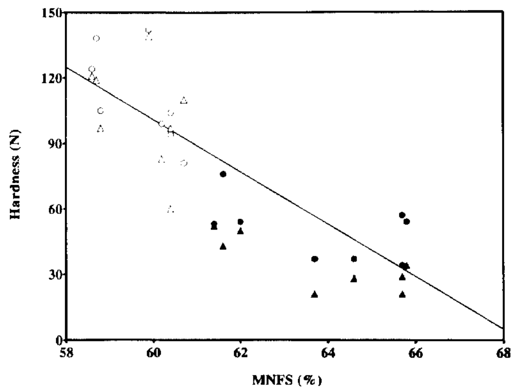

Figure 1. Hardness versus moisture in nonfat substance (MNFS) in 0-kPa Mozzarella cheeses after refrigerated storage of 1 and 6 wk. At 1 wk, hardness = −11.9 (percentage of MNFS) + 814; R^2 = .716; P = .000036. At 6 wk, hardness = −13.4 (percentage of MNFS) + 895; R^2 = .730; P = .000025; 1 wk (—) low fat (○), high fat (●); 6 wk (---) low fat (Δ), high fat (▲).

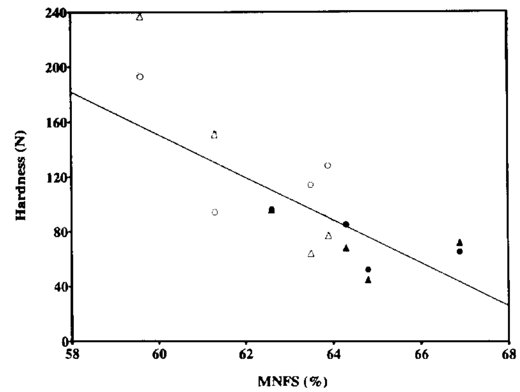

Figure 3. Hardness versus moisture in nonfat substance (MNFS) in 17,200-kPa Mozzarella cheeses after refrigerated storage of 1 and 6 wk. At 1 wk, hardness = −15.1 (percentage of MNFS) + 1059; R^2 = .548; P = .0357. At 6 wk, hardness = −24.2 (percentage of MNFS) + 1638; R^2 = .759; P = .00486; 1 wk (—) low fat (○), high fat (●); 6 wk (—) low fat (Δ), high fat (▲).

less hydration of protein, less freedom of movement for the protein molecules, larger amounts of intact caseins, and a firmer casein matrix (13, 16). Figures 1 to 3 show that high percentages of MNFS are needed to produce cheese with relatively low hardness, especially as the homogenization pressure increases.

Hardness also increased when the fat content was decreased; reduction of fat in cheese results in a denser protein network. The effect was more pronounced at the higher cooking temperature, resulting in the significant temperature × fat interaction (Table 2). Hardness was also dependent on homogenization pres-

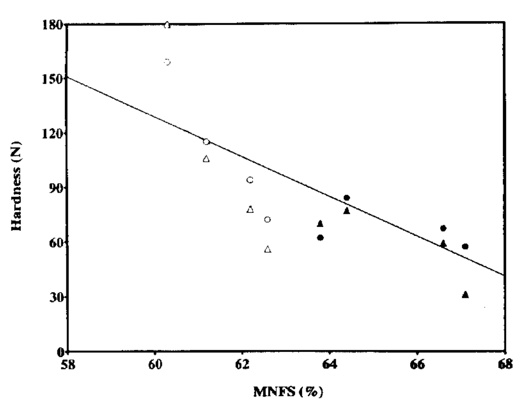

Figure 2. Hardness versus moisture in nonfat substance (MNFS) in 10,300 kPa Mozzarella cheeses after refrigerated storage of 1 and 6 wk. At 1 wk, hardness = −11.3 (percentage of MNFS) + 810; R^2 = .656; P = .0148. At 6 wk, hardness = −14.5 (percentage of MNFS) + 1006; R^2 = .619; P = .0206; 1 wk (—) low fat (○), high fat (●); 6 wk (---) low fat (Δ), high fat (▲).

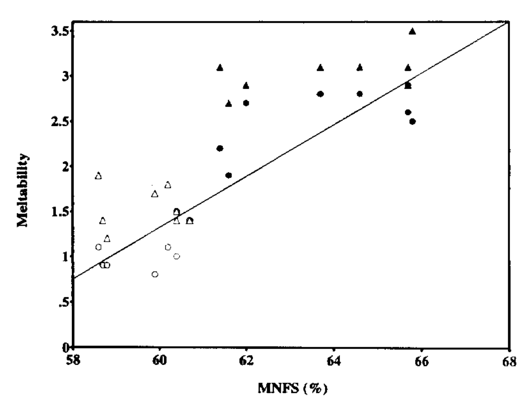

Figure 4. Meltability versus moisture in nonfat substance (MNFS) in 0=kPa Mozzarella cheeses after refrigerated storage of 1 and 6 wk. At 1 wk, meltability = .281 (percentage of MNFS) − 15.6; R^2 = .794; P = .000004. At 6 wk, meltability = .269 (percentage of MNFS) − 14.3; R^2 = .715; P = .000037; 1 wk (—) low fat (○), high fat (●); 6 wk (---) low fat (Δ), high fat (▲).

sure, particularly at the higher temperature, and temperature × pressure interaction also was significant. The fate of fat globule membranes in homogenized milk is not certain, but most of the membrane is generally assumed to be removed from the globules and replaced, at least in part, by casein micelles or submicelles (11, 15). Crosslinking of this casein with the protein matrix would make the cheese harder.

Springiness increased as fat content decreased (Table 4) because the absence of fat results in a more flexible protein network. This effect diminishes with increasing pressure, as evidenced by a significant fat × pressure interaction. Springiness decreased during storage, indicating structural breakdown in the cheese. This decrease is larger at low cooking temperature and at high fat percentages, leading to significant temperature × week and fat × week interactions (Table 2).

Meltability

Meltability increased with fat content and storage time (Table 4). Mozzarella spreads when it melts because the base of the cheese disk flows under the weight of the upper layer (15). This spread increases with fat content and also increases with proteolysis during storage because of breakdown of α_{s1}-casein, solubilization of the resulting fragments, and release of fat (8). Meltability decreased as cooking temperature and pressure increased. Higher cooking temperatures result in a lower MNFS, causing the protein matrix to become firmer and more likely to support its own weight when heated. Homogenization decreases the meltability of Mozzarella (7, 15) because adsorption of casein onto the lipid droplets apparently prevents the melted fat from spreading out. This effect is more pronounced at 6 wk and for HF cheeses, as evidenced by the significant pressure × week and pressure × fat interactions (Table 2). However, the LF, LT samples prepared from milk homogenized at 10,300 kPa melted as well as their HF counterparts, especially after 6 wk. These LF samples exhibited about as much formation of α_{s1}-I-casein as the corresponding HF samples. This result is important to the manufacture of an acceptable LF Mozzarella. Figure 4 shows the variation of the meltability of 0-kPa Mozzarella with MNFS.

Storage and Loss Moduli

The storage modulus, an indication of the ability to store energy while maintaining structural integrity, decreased with storage time because of proteolytic breakdown (Table 4). Storage modulus increased with pressure, especially with the HF cheeses, because of crosslinking of the casein that had adsorbed onto the fat globules. Storage modulus was also dependent on cooking temperature because of MNFS levels. Loss modulus, which is a measure of the ability to dissipate energy, exhibited the same trends (data not shown).

The loss tangent, also known as tan delta, is obtained by dividing loss modulus by storage modulus. The values for loss tangent were .35 ± .03 in the 0-kPa cheeses, .30 ± .02 in the 10,300-kPa cheeses, and .31 ± .03 in the 17,200-kPa cheeses. Loss tangent values in this range indicate strong structure and predominately solid behavior.

CONCLUSIONS

Homogenization of Mozzarella cheese milk at 10,300 kPa usually results in a product with greater hardness and less meltability than cheese made from nonhomogenized milk; the effects become more severe at 17,200 kPa. Cooking of Mozzarella at 32.4°C allows more moisture to be retained than at 45.9°C, resulting in more proteolysis of α_{s1}-casein during refrigerated storage. This effect decreases the springiness and elastic modulus of the samples and increases meltability. Lowering the fat content of Mozzarella increases the hardness and springiness and decreases the meltability, but an LF, 10,300-kPa cheese can have hardness, springiness, and meltability comparable with those of the HF counterpart.

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